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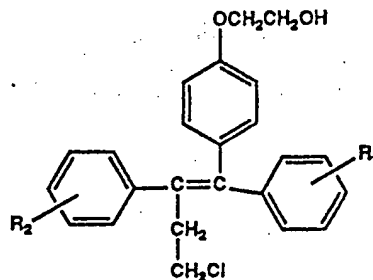
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<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>(21) International Application Number: <b>PCT/FI95/00475</b></p> <p>(22) International Filing Date: <b>6 September 1995 (06.09.95)</b></p> <p>(30) Priority Data: <b>9418067.6                      7 September 1994 (07.09.94)    GB</b></p> <p>(71) Applicant (for all designated States except US): <b>ORION-YHTYMA OY [FI/FI]; Orionintie 1, FIN-02200 Espoo (FI).</b></p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): <b>DEGREGORIO, Michael [US/US]; 7689 Shadow Oaks Lane, Granite Bay, CA 95746 (US). KANGAS, Lauri, Veikko, Matti [FI/FI]; Pasantie 3 B, FIN-21280 Raisio (FI). HÄRKÖNEN, Pirkko [FI/FI]; Vuorelantie 3 B 43, FIN-20720 Turku (FI). VÄÄNÄNEN, Kalervo [FI/FI]; Peikontie 2 B 43, FIN-90550 Oulu (FI). LAINE, Aire [FI/FI]; Sairashuoneenkatu 7 B 46, FIN-20100 Turku (FI). WIEBE, Valerie [US/US]; 7689 Shadow Oaks Lane, Granite Bay, CA 95746 (US).</b></p> <p>(74) Agent: <b>ORION CORPORATION; Orion-Farmos, Research &amp; Development, Patent Service, P.O. Box 65, FIN-02101 Espoo (FI).</b></p> </div> <div style="width: 48%;"> <p>(81) Designated States: <b>AM, AU, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LT, LV, MD, MK, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</b></p> <p><b>Published</b> <i>With international search report.</i></p> <div style="text-align: center; margin-top: 20px;">   <b>AMI INFORMATION SERVICES</b>  P.O. BOX 405, CORTE MADERA, CA 94976-0405  (415) 927-0340    FAX (415) 927-7250 </div> </div> </div>		

(54) Title: **TRIPHENYLETHYLENES FOR THE PREVENTION AND TREATMENT OF OSTEOPOROSIS**

(57) Abstract

Compounds having formula (I) wherein R<sub>1</sub> and R<sub>2</sub> are independently H or OH, are useful in the treatment and prevention of osteoporosis. The compounds of formula (I) are devoid of significant antiestrogenic and estrogenic activity.



(I)

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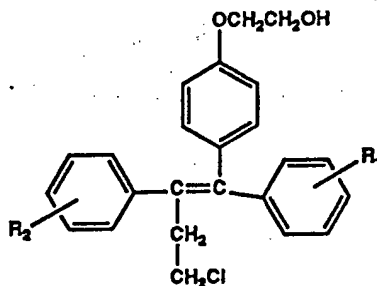
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(74) Agent: ORION CORPORATION; Orion-Farmos, Research & Development, Patent Service, P.O. Box 65, FIN-02101 Espoo (FI).			

**AML** INFORMATION SERVICES  
P.O. BOX 405, CORTE MADERA, CA 94976-0405  
(415) 927-0340 FAX (415) 927-7253

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## (57) Abstract

Compounds having formula (I) wherein R<sub>1</sub> and R<sub>2</sub> are independently H or OH, are useful in the treatment and prevention of osteoporosis. The compounds of formula (I) are devoid of significant antiestrogenic and estrogenic activity.



(I)

## TRIPHENYLETHYLENES FOR THE PREVENTION AND TREATMENT OF OSTEOPOROSIS

This invention relates to the use of certain triphenylethylene compounds, that are devoid of significant antiestrogenic and estrogenic activity, for the prevention and treatment of osteoporosis.

Osteoporosis is one of the most common chronic diseases in postmenopausal women. It is characterized by rapid bone loss after the menopause resulting in bone fractures. One of the major factors in the pathogenesis of osteoporosis is increased bone resorption in association with estrogen deficiency. Increased bone resorption results in decreased bone mass and decreased bone strength. The current preventive treatment includes the use of estrogen replacement therapy in postmenopausal women.

Estrogen and transforming growth factors- $\beta$  (TGF- $\beta$ ) are key factors in bone remodelling and may have overlapping functions. They are both capable of inducing collagen and inhibiting bone resorption. It is still unclear whether estrogen functions through a pathway that regulates TGF- $\beta$  production.

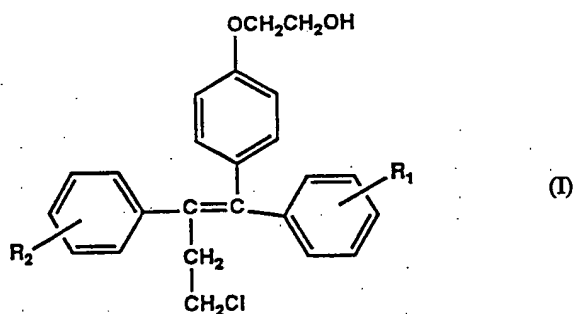
Postmenopausal bone loss occurs when coupling between bone formation and bone resorption is no longer balanced. Estrogen replacement therapy results in decreased bone turnover, decreased bone resorption and decreased fracture occurrence in postmenopausal patients. However, results of in vitro experiments have not provided clear-cut clues to the mechanism by which estrogen exerts its effects on bone. Data suggest that estrogen may act on bone by several mechanisms, either directly through estrogen receptors, indirectly through other cells besides bone cells and/or in concert with other factors and hormones such as progesterones. A long-term use of estrogens is associated with potential side effects due to the increased risk for cancers of estrogen dependent tissues such as endometrium and breast.

Triphenylethylene group antiestrogens tamoxifen and toremifene have recently been shown to stimulate TGF- $\beta$  production by fibroblasts (Colletta et al., Br. J. Cancer, 62, 405-409, 1990. Tamoxifen has also been shown to have a positive effect on bone density (Jordan et al, Breast Cancer Res. Treatm, 10, 31-35, 1987). Tamoxifen and toremifene are both in a group of compounds that are used clinically for the treatment of estrogen receptor positive breast

cancer. In patients with breast cancer these compounds primarily exert their antiestrogenic properties by blocking the estrogen receptors present in breast cancer cells, thus inhibiting cancer cell growth. Although their primary mode of action is inhibition of estrogen receptors they are also known to behave as estrogen agonists, particularly in uterine tissue in which they stimulate cell proliferation. Several reports have now suggested that tamoxifen may in fact induce secondary uterine tumors in women receiving long term tamoxifen therapy. The antiestrogenic triphenylethylenes also share many hormonal side effects in common with the estrogens, including hot flashes, nausea, menstrual irregularity and the potential for development of life-threatening thrombotic disorders.

While the clinical efficacy of tamoxifen as an agent to prevent and/or treat osteoporosis remains to be elucidated, the side effects of the antiestrogenic triphenylethylenes would be a particular disadvantage for the type of long term, chronic therapy that is required to prevent osteoporosis. On the other hand agents, that had the ability to prevent osteoporosis but were devoid of hormone related side effects such as uterine hyperplasia, hot flashes, nausea and thromboembolic complications would be very useful clinically.

According to the invention a compound having formula (I)

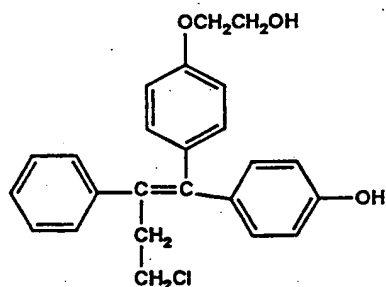


wherein  $R_1$  and  $R_2$  are independently H or OH, is used in the manufacture of a medicament for use in the prevention and treatment of osteoporosis. It has been found that the compounds of formula (I), which are devoid of significant antiestrogenic and estrogenic activity, effectively inhibit the ovariectomy-induced loss of bone mineral contents and increase bone strength in rats. It has also been discovered that the compounds of formula (I) stimulate the production of TGF- $\beta$  by bone cells. The stimulation activity of the compounds according to the invention is greater than that of tamoxifen or toremifene and

equals the activity of estrogen. Furthermore the compounds of formula (I) may enhance the useful properties of estrogen while blocking the adverse effects of estrogen on endometrial tissue when used in combination. Unlike estrogen or partial antiestrogens, the compounds of formula (I) lack significant hormone associated side effects. Therefore the compounds of formula (I) are especially suitable for use in the prevention and treatment of osteoporosis.

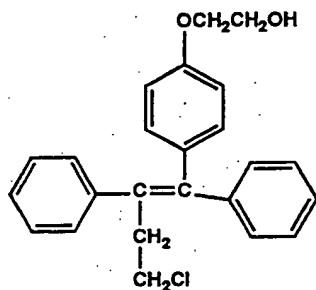
The term osteoporosis means here postmenopausal or involutional osteoporosis and other bone diseases, which are characterized by loss of bone mass.

- 10 The preferred compounds of the invention are  
 (deaminohydroxy)toremifene or 2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)-phenoxy]ethanol),  
 4-hydroxy(deaminohydroxy)toremifene or 4-[4-chloro-1-[4-(2-hydroxyethoxy)-phenyl]-2-phenyl-but-1-enyl]phenol) and  
 15 4,4'-dihydroxy(deaminohydroxy)toremifene or 4-[4-chloro-1-[4-(2-hydroxyethoxy)phenyl]-2-(4-hydroxyphenyl)but-1-enyl]phenol:

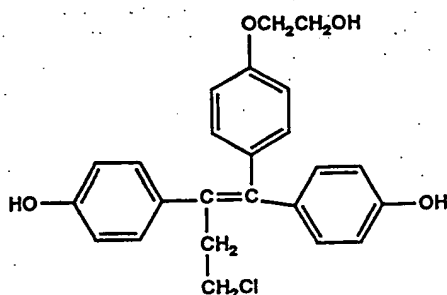


4-hydroxy(deaminohydroxy)toremifene

20



(deaminohydroxy)toremifene



4,4'-dihydroxy(deaminohydroxy)toremifene

These compounds are metabolites of toremifene and have been described earlier in Kangas L., "Biochemical and pharmacological effects of toremifene metabolites", *Cancer Chemother. Pharmacol.*, (1990), 27, 8-12 and Sipilä H. et al., "Metabolism of toremifene in the rat", *J. Steroid Biochem.*, 36, 3, 211-215, (1990). However, it has not been known that these compounds are useful for treating or preventing osteoporosis. The compounds of formula (I) can be prepared using methods known in the art.

The invention provides a method for treating or preventing osteoporosis which method comprises administering a pharmaceutically active amount of a compound of formula (I) to a patient in need of such treatment.

The compounds of the invention may be administered alone or together with other active compounds. The experimental data shows that the compounds of formula (I) may decrease the adverse effects of estrogen on endometrial tissue when used in combination. Therefore the invention also comprises the use of compounds of formula (I) together with an estrogen, such as  $\beta$ -estradiol, in treating or preventing osteoporosis.

The present invention also provides the use of a compound of formula (I) in the manufacture of a medicament for use in the prevention or treatment of osteoporosis.

The invention also provides a pharmaceutical composition for use in treating or preventing osteoporosis, which composition comprises a therapeutically effective amount of a compound of formula (I).

The compound of formula (I) may be administered in a variety of ways including orally, parenterally or transdermally using conventional forms of preparations, such as capsules, tablets, granules, powders, suppositories, injections, patches, suspensions and syrups. The compounds of the invention

may be administered monthly, weekly or daily or several times a day depending upon the patient's needs. A typical daily oral dosage is within the range of from about 0.5 mg to about 1000 mg, preferably from about 1 mg to about 500 mg, more preferably from about 5 mg to about 100 mg, of the active compound. However, the dosage may be properly varied depending on the age, body weight and conditions of the patient as well as on the administration method.

The compositions according to the invention can be prepared by the methods commonly employed in the art. In addition to the active compound the compositions may contain pharmaceutically acceptable additives commonly used in the art, such as carriers, binders, excipients, lubricants, suspending agents and diluents. The amount of the active compound in the compositions of the invention is sufficient to produce the desired therapeutical effect, for example about 0.5 to 1000 mg, preferably about 1 mg to 500 mg, more preferably about 5 mg to 100 mg, in unit dosage for both oral and parenteral administration.

The following examples illustrate the synthesis of the compounds of the invention.

#### EXAMPLES

##### Example 1.

Z-4-[4-(2-benzyloxyethoxy)-phenyl]-3,4-diphenyl-but-3-en-1-ol

The reaction vessel was charged with toluene (790 ml), 48 % aqueous sodium hydroxide (790 ml), tetrabutylammonium bromide (2.12 g, 6.6 mmol) and Z-4-(4-hydroxy-1,2-diphenyl-but-1-enyl)-phenol (50 g, 0.16 mol) prepared by the method described by United States Patent 4,996,225. The mixture was refluxed for 30 minutes. Benzyl-(2-bromoethyl)ether (Grobely D. et al., Tetrahedron Letters 28, 2639-42, 1979) (41.7 g, 0.19 mol) was added to the reaction mixture and the refluxing was continued for 2 hours. Then the mixture was cooled to room temperature, layers were separated and aqueous phase was washed with toluene. Toluene phases were combined, washed with water, dried and evaporated to dryness. The residue was used in the next stage without further purification.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): d 1.2 (1H, t, OH), 2.8 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.6 (2H, dt,  $\text{CH}_2\text{OH}$ ), 3.7 (2H, t,  $\text{CH}_2\text{OBn}$ ), 4.0 (2H, t,  $\text{CH}_2\text{OPh}$ ), 4.6 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 6.6 (2H, d, H-PhO), 6.8 (2H, d, H-PhO), 7.1-7.4 (15H, m, H-Ph).

#### Example 2.

5        Z-1-[4-(2-benzyloxyethoxy)-phenyl]-4-chloro-1,2-diphenyl-but-1-ene

      Z-4-[4-(2-benzyloxyethoxy)-phenyl]-3,4-diphenyl-but-3-en-1-ol prepared in the previous stage was dissolved in acetonitrile (400 ml). Triphenyl phosphine (103.5 g, 0.4 mol) and tetrachloromethane (120 g, 0.79 mol) were added and the mixture was refluxed for 2 hours. Then the mixture  
10        was evaporated to dryness under reduced pressure. The residue was dissolved in methanol (160 ml) and water (40 ml) and extracted three times with petroleum ether (3 x 200 ml) at boiling point. Petroleum ether layers were combined and evaporated to dryness under reduced pressure. The residue was crystallized twice from ethanol (700 ml). Yield 36 g.

15         $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): d 2.9 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.4 (2H, dt,  $\text{CH}_2\text{Cl}$ ), 3.7 (2H, t,  $\text{CH}_2\text{OBn}$ ), 4.0 (2H, t,  $\text{CH}_2\text{OPh}$ ), 4.6 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 6.6 (2H, d, H-PhO), 6.8 (2H, d, H-PhO), 7.1-7.4 (15H, m, H-Ph).

#### Example 3.

      Z-2-[4-(4-chloro-1,2-diphenyl-but-1-enyl)-phenoxy]-ethanol

20        Z-1-[4-(2-benzyloxyethoxy)-phenyl]-4-chloro-1,2-diphenyl-but-1-ene (36 g, 0.08 mol) was dissolved in the mixture of ethyl acetate (350 ml) and ethanol (350 ml). Palladium on carbon (5%, 0.28 g) was added and the solution was flushed with hydrogen gas until there was not any starting compound left (thin layer chromatography). Palladium on carbon was filtered  
25        off through siliceous earth and the filtrate was evaporated. The residue was crystallized from the mixture of ethanol (155 ml) and water (65 ml). Yield 20 g.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): d 2.9 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.4 (2H, dt,  $\text{CH}_2\text{Cl}$ ), 3.84-3.89 (2H, m,  $\text{CH}_2\text{OH}$ ), 3.92-3.96 (2H, m,  $\text{CH}_2\text{OPh}$ ), 6.6 (2H, d, H-PhO), 6.8 (2H, d, H-PhO), 7.1-7.4 (10H, m, H-Ph).

#### 30        Example 4.

      Z-4-[4-(2-benzyloxyethoxy)-phenyl]-4-(4-benzyloxyphenyl)-3-phenyl-but-3-en-1-ol



The reaction vessel was charged with sodium hydride (0.43 g, 0.0178 mol) and dimethyl formamide (34 ml). 4-[1-(4-Benzyloxyphenyl)-4-hydroxy-2-phenyl-but-1-enyl]phenol (5 g, 0.0118 mol) prepared by the method of United States Patent 4,996,225 was dissolved to dimethyl formamide (6 ml), added to the mixture at room temperature and stirring was continued for half an hour. Then benzyl-(2-bromoethyl)ether (3.06 g, 0.014 mol) was added dropwise during 20 minutes to the reaction mixture and stirring was continued for additional 4 hours. The reaction mixture was poured into brine and extracted with ethyl acetate. Organic layer was washed with 2 N hydrogen chloride solution and twice with water, dried and evaporated to dryness. The product was purified by flash chromatography (toluene: methanol, 9.8:0.2). Yield 2.8 g.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): d 2.78 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.59 (2H, t,  $\text{CH}_2\text{OH}$ ), 3.83-3.86 (2H, m,  $\text{CH}_2\text{OBn}$ ), 4.15-4.19 (2H, m,  $\text{CH}_2\text{OPh}$ ), 4.65 (2H, s,  $\text{CH}_2\text{OCH}_2\text{Ph}$ ), 6.79 (2H, d, H-PhO), 6.9 (2H, d, H-PhO), 7.11-7.37 (17H, m, H-PhO, H-Ph).

#### Example 5.

Z-1-[4-(2-benzyloxyethoxy)-phenyl]-1-(4-benzyloxyphenyl)-4-chloro-2-phenylbut-1-ene

4-[4-(2-benzyloxyethoxy)-phenyl]-4-(4-benzyloxyphenyl)-3-phenylbut-3-en-1-ol (1.9 g, 3.45 mmol), triphenyl phosphine (1.8 g, 6.9 mmol), carbon tetrachloride (2.6 g, 17.25 mmol) and acetonitrile (10 ml) were stirred at room temperature for an hour. The product was separated by the same method as Z-1-[4-(2-benzyloxyethoxy)-phenyl]-4-chloro-1,2-diphenyl-but-1-ene described in example 2 and purified by flash chromatography (toluene : methanol, 9.75:0.25). Yield 1.2 g.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): d 2.96 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.42 (2H, t,  $\text{CH}_2\text{Cl}$ ), 3.72-3.75 (2H, m,  $\text{CH}_2\text{OBn}$ ), 3.99-4.02 (2H, m,  $\text{CH}_2\text{OPh}$ ), 4.58 (2H, s,  $\text{CH}_2\text{OCH}_2\text{Ph}$ ), 5.07 (2H, s,  $\text{PhOCH}_2\text{Ph}$ ), 6.57 (2H, d, H-PhO), 6.77 (2H, d, H-PhO), 6.98 (2H, d, H-PhO), 7.10-7.47 (17H, m, H-Ph, H-PhO).

#### Example 6.

Z-4-[4-chloro-1-[4-(2-hydroxyethoxy)phenyl]-2-phenyl-but-1-enyl]-phenol

1-[4-(2-Benzoyloxyethoxy)-phenyl]-1-(4-benzoyloxyphenyl)-4-chloro-2-phenylbut-1-ene (1.0 g, 1.74 mmol) was debenzylated by the same method as Z-1-[4-(2-benzoyloxyethoxy)-phenyl]-4-chloro-1,2-diphenyl-but-1-ene described in example 3. The residue was crystallised from the mixture of ethanol and water (1:1). Yield 0.3 g.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ): d 2.95 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.42 (2H, t,  $\text{CH}_2\text{Cl}$ ), 3.86-3.91 (2H, m,  $\text{CH}_2\text{OH}$ ), 3.94-3.97 (2H, m,  $\text{CH}_2\text{OPh}$ ), 6.56 (2H, d, H-PhO), 6.78 (2H, d, H-PhO), 6.83 (2H, d, H-PhO), 7.11-7.22 (7H, m, H-Ph, H-PhO).

#### Example 7.

10 Z,E-4-[4-(2-benzoyloxyethoxy)phenyl]-3,4-bis-(4-benzoyloxyphenyl)-but-3-en-1-ol

4-[1,2-Bis-(4-benzoyloxyphenyl)-4-hydroxy-but-1-enyl]phenol (2 g, 3.8 mmol) (prepared from 4-benzoyloxy-4'-tetrahydropyranyloxy benzophenone and 4-benzoyloxycinnamic acid ethyl ester by the method described in United States Patent 4,996,225, potassium carbonate (1.56 g, 1.13 mmol), KI (catalytic amount) and ethanol (40 ml) were refluxed for 15 min. Benzyl-(2-bromoethyl)ether (Grobely D. et al., Tetrahedron Letters 28, 2639-42, 1979) (1.1 g, 4.6 mmol) was added dropwise to the reaction mixture during half an hour and refluxing was continued for additional 2 hours. Then extra portion of 20 potassium carbonate (0.78 g, 0.57 mmol) and benzyl-(2-bromoethyl)ether (0.5 g, 2.3 mmol) was added to the reaction mixture and refluxing was continued for 2 hours. After completion of the reaction the mixture was filtered, filtrate was evaporated to dryness under reduced pressure and the residue was purified by flash chromatography (toluene : methanol, 9.9:0.1). Yield 2.2 g.

25  $^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3 + \text{CD}_3\text{OD}$ ): d 2.75 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.57 (2H, t,  $\text{CH}_2\text{OH}$ ), 3.75-3.91 (2H, m,  $\text{CH}_2\text{OBn}$ ), 4.01-4.23 (2H, m,  $\text{CH}_2\text{OPh}$ ), 4.58 and 4.64 (2H, s,  $\text{CH}_2\text{OCH}_2\text{Ph}$ ), 4.93 and 5.0 and 5.1 (4H, s,  $\text{PhOCH}_2\text{Ph}$ ), 6.5-7.45 (27H, m, H-PhO, H-Ph).

#### Example 8.

30 Z,E-1-[4-(2-benzoyloxyethoxy)phenyl]-1,2-bis-(4-benzoyloxyphenyl)-4-chloro-but-1-ene

1-[4-(2-benzoyloxyethoxy)phenyl]-1,2-bis-(4-benzoyloxyphenyl)-4-chloro-but-1-ene was prepared from 4-[4-(2-benzoyloxyethoxy)phenyl]-3,4-bis-(4-

benzyloxyphenyl)-but-3-en-1-ol (0.65 g, 0.98 mmol), triphenyl phosphine (0.5 g, 1.96 mmol) and carbon tetrachloride (0.74 g, 4.9 mmol) by the method described in example 2. The product was purified by flash chromatography (toluene). Yield 0.42 g.

- 5         $^1\text{H}$  NMR (80 MHz,  $\text{CDCl}_3 + \text{CD}_3\text{OD}$ ): d 2.92 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.43 (2H, t,  $\text{CH}_2\text{Cl}$ ), 3.74-4.18 (4H, m,  $\text{OCH}_2\text{CH}_2\text{O}$ ), 4.59 and 4.64 (2H, s,  $\text{CH}_2\text{OCH}_2\text{Ph}$ ), 4.93 and 5.0 and 5.07 (4H, s,  $\text{PhOCH}_2\text{Ph}$ ), 6.6-7.5 (27H, m, H-PhO, H-Ph).

#### Example 9.

- 10        Preparation of Z,E-4-[4-chloro-1-[4-(2-hydroxyethoxy)phenyl]-2-(4-hydroxyphenyl)but-1-enyl]phenol

- 15        1-[4-(2-benzyloxyethoxy)phenyl]-1,2-bis-(4-benzyloxyphenyl)-4-chloro-but-1-ene (0.4 g, 0.66 mmol) was debenzylated by the method described in example 3 despite of the temperature, which was  $40^\circ\text{C}$  in this reaction. The product was purified by flash chromatography (toluene : methanol, 9.4:0.6). Yield 0.18 g.

$^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ): d 2.86 and 2.88 (2H, t,  $\text{CH}_2\text{-C=}$ ), 3.41 (2H, t,  $\text{CH}_2\text{Cl}$ ), 3.77-4.07 (4H, m,  $\text{OCH}_2\text{CH}_2\text{OH}$ ), 6.44 (2H, d, H-PhO), 6.58-7.17 (12H, m, H-PhO).

- 20        The following experiments illustrate the effects of the compounds of the invention.

### EXPERIMENTS

#### Effects on the production of TGF $\beta$

- 25        Analysis of latent TGF $\beta$  produced by osteoblast derived osteosarcoma cells (ROS 17/2.8) cells and UMR-106 cells were performed by analysis of mRNA using northern blot analysis. In addition, different forms of TGF $\beta$  were analyzed by HPLC, western analysis and immuno-precipitation. The results are shown in Table 1. Clearly, compounds A and C are very active in producing the release of TGF $\beta$  and appear to be as active as estrogen.

30

Compound A = (deaminohydroxy)toremifene

Compound C = 4-hydroxy(deaminohydroxy)toremifene

Table 1. Effects of  $\beta$ -estradiol, the antiestrogens tamoxifen and toremifene, and compounds A and C on the production of latent TGF $\beta$  by osteosarcoma cells ROS 17/2.8 and UMR-106.

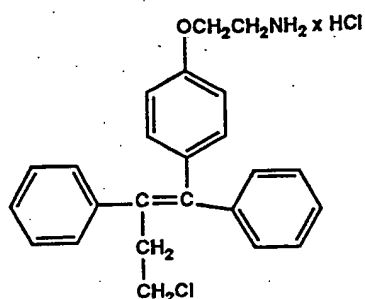
Cell line	Agents	10 <sup>-7</sup> M
ROS 17/2.8	$\beta$ -estradiol	1.1
	tamoxifen	0.6
	toremifene	0.6
	compound A	1.6
	compound C	1.7
UMR-106	$\beta$ -estradiol	1.8
	tamoxifen	1.1
	toremifene	1.3
	compound A	1.5
	compound C	1.6

Data is expressed as treated/control ratios.

All determinations are the average of triplicate samples.

Effects on the growth of estrogen receptor positive and negative breast cancer cells

This experiment was performed to prove that the concentrations that produce latent TGF $\beta$  by the osteoblast-like cells by compounds A and C are independent of hormone action. Breast cancer cell lines MCF-7 ER+ and MDA-MB-231 ER- were used in the experiment. Also a negative control, compound B, was used. Compound B has the essential amine group that has been shown as necessary for antiestrogenic activity (see Jordan et al., "Importance of the alkylaminoethoxy side-chain for the estrogenic and antiestrogenic actions of tamoxifen and toremifene in the immature rat uterus", Molecular and Cellular Endocrinology, 27, 291-306, 1982). Although complete removal of the alkylaminoethoxy side-chain will produce an estrogen like compound, compounds A and C are stable and not susceptible to this cleavage.



Compound B (control)

- 5 Table 2 shows the results of exposing estrogen, compound A, B, C, and combinations of both when exposed to the estrogen receptor positive breast cancer cells MCF-7. The results show that estrogen alone appeared to stimulate the growth of cells whereas compounds A, B and C lack such activity. Clearly, control compound B has an antiestrogen effect that is partially blocked by estrogen. Compounds A and C had little effect on cells alone and a net stimulatory activity similarly to estrogen alone when used in combination with
- 10 estrogen. This result suggest that compounds A and C at  $10^{-7}$  M had not significant antiestrogenic activity.

15 All compounds had little to no effect on the growth of estrogen receptor negative breast cancer cells MDA-MB-231.

Table 2. Effect of compounds A, B, C  $\pm$  Estradiol (E2) on MCF-7 ER+

Treatment	Percent Survival $\pm$ SD
Control	100.00
E2 ( $10^{-9}$ M) control	104.62 $\pm$ 0.72
Compound A ( $10^{-7}$ M)	89.68 $\pm$ 3.91
Compound A + E2	97.70 $\pm$ 4.62
Compound B ( $10^{-7}$ M)	47.22 $\pm$ 4.04
Compound B + E2	90.73 $\pm$ 3.07
Compound C ( $10^{-7}$ M)	85.57 $\pm$ 2.07
Compound C + E2	99.80 $\pm$ 4.90

20 The ability of compound A to inhibit the antiestrogenic activity of tamoxifen was also studied. The results (TABLE 3) show that compound A does not inhibit the antiestrogenic activity of tamoxifen. This, together with the

25 fact that the compounds of the invention did not stimulate or inhibit MCF-7

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growth, is a further evidence of a non-hormonal mechanism of the compounds of the invention.

TABLE 3. MCF-7 growth inhibition by tamoxifen and tamoxifen + compound A

5	Tamoxifen ( $\mu\text{M}$ )	Percent survival
	0.1	89.1
	0.5	53.6
	1.0	46.1
10	2.0	43.8
	5.0	27.2
	Tamoxifen ( $\mu\text{M}$ ) + A ( $10^{-7}$ M)	Percent survival
	0.1	80.4
15	0.5	56.9
	1.0	50.6
	2.0	40.9
	5.0	23.7

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#### Uterine effects

This study examined the uterine effects of compounds A, B, C and estrogen in the ovariectomized rat. It was further examined whether compound A could effectively block the uterotrophic effects of estrogen.

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40 Sprague-Dawley female rats, 92 days old, were used. Bilateral ovariectomies were performed after anesthetizing the animals with metaphane. Sham surgery was performed by exteriorizing the ovaries but not removing them. The compounds were delivered in a vehicle of 95% corn oil and 5% benzyl alcohol by subcutaneous injection at 250  $\mu\text{l/day}$ . All treatments were initiated the first day after surgery for 21 days. The animals were also injected for fluorochrome labeling at 14 days before sacrifice and tetracycline hydrochloride (25 mg/kg) at 6 days before sacrifice. The pre and post weights of the animals were taken and the animals exsanguinated. The uteri were removed and weighed. The bones were fixed in 70 % ethanol for histological examination. The blood, uteri and one humerus from each animal were assayed for hormone levels and one tibia for histological quantitation of trabecular bone volume.

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Table 4 shows that the uterotrophic effects of estrogen were counteracted by compound A. This is new evidence that a non-antiestrogenic agent, which can not be metabolized to an antiestrogenic compound can effectively

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block the adverse effects of estrogen on the uterus. It is very likely that TGF $\beta$  may inhibit the uterine growth despite the estrogenic properties of estrogen.

Table 4. Effect of compounds A, B, C  $\pm$  Estradiol (E2) on rat uterus

5	Treatment	Average uterine weight (g) $\pm$ SD (n=6)
10	Sham control	0.7176 $\pm$ 0.0896
	E2 control	0.7140 $\pm$ 0.1560
	Compound A	0.3112 $\pm$ 0.0460
	Compound A + E2	0.3540 $\pm$ 0.0600
	Compound B	0.3270 $\pm$ 0.0840
15	Compound C	0.3656 $\pm$ 0.0450

#### Effects on bone

The inhibitory effect of compound A on the ovariectomy-induced loss of bone mineral contents was demonstrated by measuring the ash weight of the epiphysis of rat tibia and by determining the volume of trabecular bone in distal femur by morphometric analysis. The results are presented in Table 5 and Figure 1. The treatment groups 1-10 of Figure 1 are the same as those in Table 5.

Table 5. Effect of compound A on the ash weight of rat left tibia

25	Treatment (4 weeks as indicated)	Ash weight (mg) n=10
30	1. Normal control 2. + 17 $\beta$ -E2 (sc, 30-day pellet) 3. + Compound A 1 mg/kg/day (po) 4. + Compound A 10 mg/kg/day (po)	284.2 $\pm$ 13.7 * 295.0 $\pm$ 17.7 * 287.9 $\pm$ 12.1 * 292.0 $\pm$ 13.6 *
35	5. Ovariectomized control 6. + 17 $\beta$ -E2 (sc) 7. + Compound A 1 mg/kg/day 8. + Compound A 1 mg/kg/day + 17 $\beta$ -E2 9. + Compound A 10 mg/kg/day 40 10. + Compound A 10 mg/kg/day + 17 $\beta$ -E2	259.9 $\pm$ 14.6 293.2 $\pm$ 20.0 * 263.9 $\pm$ 16.6 * 279.6 $\pm$ 16.9 * 276.3 $\pm$ 15.9 * 285.5 $\pm$ 15.6 *

\* significantly different from ovariectomized control,  $p < 0.05$

The beneficial effect of compound A on trabecular bone mineral contents and volume proportion were associated with increased bone

strength in femoral neck and lumbar vertebrae. This was demonstrated by measuring resistance against torsion and compression, respectively (Table 6).

Table 6. Effect of compound A on the mechanical strenght of rat femoral neck and L4 vertebra

5	Treatment (4 weeks as indicated)	Strength of femoral neck (N)	Compression of L4 (N)
10	Normal control	106.2 ± 18.3 <sup>1)</sup>	516.8 ± 75.6
	+ 17β-E2 (sc, 30-day pellet)	101.9 ± 20.0	657.3 ± 64.0 <sup>3,4)</sup>
	+ A 1 mg/kg/day (po)	103.1 ± 14.6 <sup>2)</sup>	619.6 ± 114.4 <sup>3,4)</sup>
	+ A 10 mg/kg/day (po)	101.0 ± 12.3	628.9 ± 108.7 <sup>3,4)</sup>
15	Ovarectomized control	86.8 ± 13.4	449.7 ± ±83.0
	+ 17β-E2 (sc)	120.7 ± 18.1 <sup>3)</sup>	582.7 ± 91.8 <sup>2)</sup>
	+ A 1 mg/kg/day	87.8 ± 15.2	592.4 ± 64.0 <sup>3)</sup>
	+ A 1 mg/kg/day + 17β-E2	100.2 ± 20.5	619.6 ± 82.1 <sup>3)</sup>
	+ A 10 mg/kg/day	103.7 ± 12.7 <sup>1)</sup>	549.4 ± 81.8 <sup>1)</sup>
	+ A 10 mg/kg/day + 17β-E2	115.1 ± 18.3 <sup>2)</sup>	641.8 ± 64.6 <sup>3,5)</sup>
20	<sup>1)</sup> Significantly different from ovarectomized control, p<0.05		
	<sup>2)</sup> Control, p<0.01		
	<sup>3)</sup> Control, p<0.001		
	<sup>4)</sup> Significantly different from normalcontrol, p<0.001		
25	<sup>5)</sup> Significantly different from ovarectomized control treated with A 10 mg/kg/day p<0.05		

In another in vivo study using ovariectomized rat model it was found that compound A was as effective as β-estradiol at preventing bone loss while having little effect on the uterus. When compound A was administered in combination with β-estradiol, a greater increase in percent trabecular bone volume were observed when compared to either compound alone and compound A effectively blocked the effects of estrogen on the uterus. The result of this study is presented in Table 7.

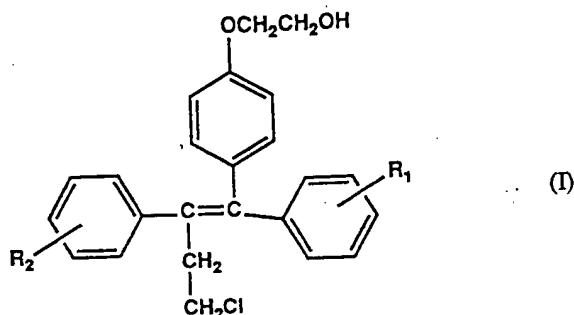
Table 7. Inhibition of ovariectomy-induced bone loss by compounds A, B, estradiol, and compound A plus estradiol.

35	Treatment group	% Trabecular bone volume (n=3)
40	compound A	25.24 ± 6.61
	compound B	8.18 ± 5.83
	Estradiol	22.6 ± 5.56
	A + E	38.4 ± 8.14



## Claims

1. Use of a compound of formula (I)



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wherein  $R_1$  and  $R_2$  are independently H or OH, in the manufacture of a medicament for use in the prevention or treatment of osteoporosis.

2. Use according to claim 1 in which the medicament comprises an estrogen.

3. A pharmaceutical composition for use in the prevention or treatment of osteoporosis comprising a therapeutically effective amount of a compound of formula (I).

4. A composition according to claim 3 which comprises a pharmaceutically acceptable carrier.

5. A composition according to claim 3 or 4 which comprises an estrogen.

6. A method for the prevention or treatment of osteoporosis which method comprises administering a pharmaceutically active amount of a compound of formula (I) to a patient in need of such treatment.

7. A method for the prevention or treatment of osteoporosis which method comprises administering a pharmaceutically active amount of a compound of formula (I) and an estrogen to a patient in need of such treatment.

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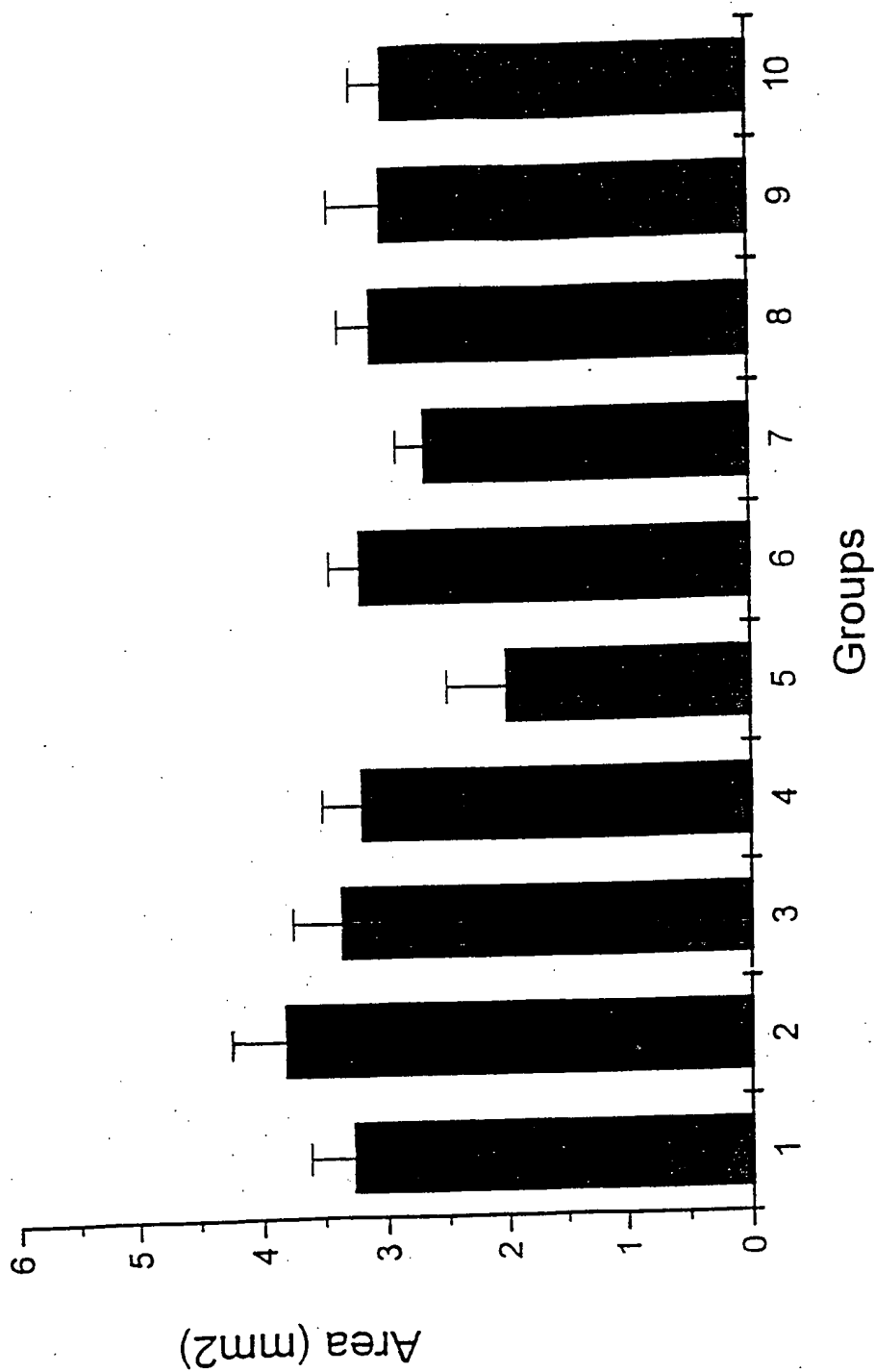


FIG. 1

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/FI 95/00475

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K31/075 A61K31/085

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER CHEMOTHERAPY AND PHARMACOLOGY, vol. 27, no. 1, 1990 pages 8-12, L. KANGAS 'BIOCHEMICAL AND PHARMACOLOGICAL EFFECTS OF TOREMIFENE METABOLITES' cited in the application	3-5
Y	see the whole document ---	1,2,6,7
Y	ACTA ONCOLOGICA, vol. 31, no. 2, 1992 pages 143-146, L. KANGAS 'AGONISTIC AND ANTAGONISTIC EFFECTS OF ANTIESTROGENS IN DIFFERENT TARGET ORGANS' see abstract see page 144, column 2, paragraph 2 - page 145, column 1, paragraph 1; table 2 ---	1,2,6,7
-/-		

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \* "A" document defining the general state of the art which is not considered to be of particular relevance
- \* "E" earlier document but published on or after the international filing date
- \* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \* "O" document referring to an oral disclosure, use, exhibition or other means
- \* "P" document published prior to the international filing date but later than the priority date claimed

- \* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \* "&" document member of the same patent family

Date of the actual completion of the international search

14 November 1995

Date of mailing of the international search report

24.11.95

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
TJ, (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Hoff, P

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/FI 95/00475

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER CHEMOTHERAPY AND PHARMACOLOGY, vol. 30, no. 3, 1992 pages 174-178, J. BISHOP ET AL. 'PHASE I CLINICAL AND PHARMACOKINETICS STUDY OF HIGH-DOSE TOREMIFENE IN POSTMENOPAUSAL PATIENTS WITH ADVANCED BREAST CANCER' see the whole document ---	3,4
A	THE JOURNAL OF STEROID BIOCHEMISTRY, vol. 36, no. 3, 1990 pages 197-202, N.H. SIMBERG ET AL. 'IN VITRO AND IN VIVO BINDING OF TOREMIFENE AND ITS METABOLITES IN RAT UTERUS' see the whole document ---	1-7
A	BREAST CANCER RESEARCH AND TREATMENT, vol. 10, no. 1, 1987 pages 31-35, V.C. JORDAN ET AL. 'EFFECTS OF ANTI-ESTROGENS ON BONE IN CASTRATED AND INTACT FEMALE RATS' cited in the application see the whole document -----	1-7

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 95/00475

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 6-7 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.